

24

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE The mutant contd

From Page N

### Annealing & Synthesis

#### Annealing

(D=A)

1.0  $\mu$ l 2899  
1.0  $\mu$ l Ann. Buff  
5.0  $\mu$ l ssDNA  
3.0  $\mu$ l H<sub>2</sub>O

(F → Tyr)

1.0  $\mu$ l 2904  
1.0  $\mu$ l Ann. Buff  
5.0  $\mu$ l ssDNA  
3.0  $\mu$ l H<sub>2</sub>O

Control  
Fibro

1.0  $\mu$ l  
1.0  $\mu$ l  
5.0  $\mu$ l s  
3.0  $\mu$ l i

75°C for 2 min → quick chill in ice water → ice

Synthesis Add

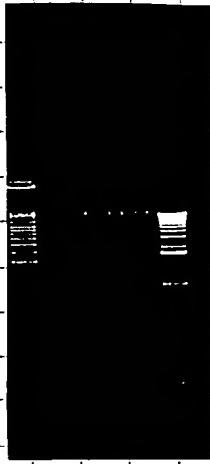
0.25  $\mu$ l T7 Pol (Sequence)

0.5  $\mu$ l T4 ligase (LTI)

5' on ice, 5' at room temp 35 min at 37°C

Add 90  $\mu$ l (10 mM EDTA + 10 mM Tris) → ice.

Load 15  $\mu$ l in agarose gel.



The synthesis looks alright. But I have some concern because the reaction did not produce clear cut band.

Any way proceed with free transformation dil sample (1:25).

Dilution of FQ 100  $\mu$ l

2  $\mu$ l 2899 or 2904 or control

25' on ice, 40' at 42°C → 30 Sec. on ice

Overlay with top agar (10  $\mu$ l and 90  $\mu$ l sample)

37°C 0/5

T Pag N

Witnessed & Understood by me,

Date

Invented by

Date

4/6/95

Recorded by

3/29/95